Atherosclerosis in Exotic Carnivora and Pinnipedia

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STUDIES OF SPONTANEOUS ATHEROSCLEROSIS in animals, although numerous, have by no means been exhaustive. 1-6 Members of the Order Primates have undergone the most thorough scrutiny, 7-15 vet many nonhuman primate species remained unexamined. Members of other orders have seldom been subjected to systematic study, and most of the available information concerning comparative atherosclerosis in these individuals has come from surveys of zoo animals, domestic animals, and occasionally wild animals in the free state. 1-6, 16-30 A remarkable dichotomy exists between the type and extent of atherosclerotic lesions present in the average elderly United States male, and those which may be found in any animal of comparable age. Since most investigators have tended to view atherosclerosis as a disorder with a single basic etiology, this dichotomy has made it difficult to compare the spontaneous arterial lesions of animals with those of man. This is unfortunate because the arterial lesions of animals are often quite similar to the early human proliferative atherosclerotic lesions. Since the arteries of most mammalian species (including man) resemble each other morphologically, it seems reasonable to assume that the finding of similar arterial lesions in different species might imply that similar pathogenic mechanisms had been operative in their production. This argument will be expanded later.

The present report is the continuation of a study of arterial disease among captive wild animals dying in the Oklahoma City Zoo. The lesions in Pinnipedia are described in some detail, since previous surveys of arterial disease in these animals are rare.⁵ The medionecrosis observed in 2 bears is also discussed at length for the same reason. Findings from the present study and from previous studies are compared and analyzed in an attempt to explain some of the differences which are seen between atherosclerosis in various animal species and in man.

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Materials and Methods

Materials were obtained from 520 consecutive necropsies performed upon mammals and birds dying in the Oklahoma City Zoo between 1964 and 1968. Aortas were flattened on cardboard, fixed in 10% formalin, stained with a dilute solution of Sudan IV, and graded for atherosclerotic involvement by two observers according to a modification ³¹ of the method of Holman *et al.*³² Portions of various lesions were removed, quick-frozen and stained with Oil Red O-hematoxylin. The remainder of each block was then embedded in paraffin and contiguous sections were cut and stained with H & E and Weigert's elastic stain. Other stains, primarily Toluidine blue or PTAH, were employed as needed.

Results

Fifty-six of the 520 individuals examined belonged to the Orders Carnivora or Pinnipedia. The findings in these 56 mammals constitute the basis of this report. The entire aorta was available for study in all but 11 of the 56 animals. In these 11, the entire thoracic aorta, plus variable portions of the abdominal aorta were present. Seventeen of the animals were less than 6 weeks of age, 6 were completely mature or elderly, and the remainder were juvenile or young adults. Precise age was frequently unknown. The sexes were nearly evenly divided, with 28 males, 24 females, and 4 unrecorded (newborn animals). Various abnormalities of the aorta were found in 27 of the 56 specimens. None of the aortic lesions was large enough to produce clinically significant luminal stenosis (Table 1).

Lesions were most prevalent in the aortas of seals (Phoca vitulina) and sea lions (Zalophus californianus), and except for being practically devoid of stainable lipid, they most resembled those of man. In the affected animals, triangular shaped pearly plaques were present distal to most intercoastal branch orifices, and larger, irregularly shaped gray intimal plagues were noted on the anterior walls of the lower thoracic and abdominal aortas. In one animal the abdominal aortic lesions consisted of clusters of small papules, and in another animal, similar papules appeared to be coalescing to form the larger irregularly shaped plaques which were seen in most of the specimens. Microscopically, the plaques appeared to be composed entirely of smooth muscle cells and contained numerous fine elastic fibers (Fig 1). The smooth muscle cells usually were oriented longitudinally in the deeper portions of the lesions, and circumferentially in the portions adjacent to the lumen. In the thicker lesions, this difference in alignment of cells occasionally resulted in the formation of two distinct layers. The internal elastic lamella was found to be almost intact beneath the smaller lesions, and focally fragmented beneath the more confluent intimal thickenings. Beneath the larger plaques, it was nearly always absent (Fig 2). The process was invariably

Table 1.

Common name	Scientific name	No. of animals		% of aorta containing lesions		
		Total	With lesions	Fatty streaks	Fibrous plaques	Others
ORDER: CARNIVORA						
Coyote	Canis latrans	5	2		tr‡, 1	1*
American	Ursus	5	5	tr, tr, tr	5, 15,	5†, 20†
black bear	americanus				20, 35	
Sloth bear	Melursus ursinus	1	1	5	1	
Cacomistle	Bassariscus astutus	3	2	tr	2	1•
Raccoon	Procyon lotor	1	1		tr	
African civet	Viverra civetta	1	1	4		
Suricate	Suricata suricatta	1				
Spotted hyena	Crocuta crocuta	1	1	2	2	
Fisher	Martes pennanti	1				
Black leopard	Panthera pardus	2				
Tiger	P. tigris	3				
Lion	P. leo	7				
Leopard cat	Felis bengalensis	1				
Fishing cat	F. viverrina	1				
Golden cat	F. temmincki	4	2		tr, 3	2*
Bobcat	F. rufa	1			•	
Spotted cat	F. rubiginosa	1	1		15	5*
Mountain lion	F. concolor	3				
American badger	Taxidea taxus	1				
Weimeraner dog	Canis familaris	2	2		tr, 1	
Total		45	18	7	15	5
ORDER: PINNIPEDIA						
Sealion	Zalophus	7	6		tr, 2, 5, 10	,
	californianus				10, 11	
Harbor Seal	Phoca vitulina	4	3		tr, 1, 5	
Total		11	9		9	

[•] parasitic lesions

more extensive and more pronounced in the abdominal aortas; the arch was usually spared. No abnormalities of the medial or adventitial layers were found.

The abnormalities found in the aortas of the Carnivora were much more variable in structure and location than those of the Pinnipedia. In 3 animals—1 coyote (Canis latrans), 1 spotted cat (Felis rubiginosa), and 1 cacomistle (Bassariscus astutus)—the predominant lesions clearly were due to migrating parasites. These were all located in the arch and upper thoracic portions of the aortas; they were gray, irregular and sometimes serpiginous in shape, Sudan negative, and not related to branch orifices. Microscopically, there was focal absence of medial smooth muscle cells, with separation of adjacent elastic fibers to form circular or

[†] saccular aneurysms ‡ trace

split-like spaces. These spaces contained either intact parasites, inflammatory cells (lymphocytes, plasma cells, polymorphonuclear leukocytes, eosinophiles, giant cells), hemosiderin laden macrophages or foam cells. The cytoplasm of the foam cells was red after staining with Oil Red-O; the nuclei could not be definitely identified. Areas interpreted as healed parasitic tracts sometimes consisted of bundles of ordinary smooth muscle cells, oriented in different planes than the adjacent medial cells. However, it was difficult to be sure that these bundles were related to previous parasitic infestation. Infiltration of the surrounding intima, media, and adventitia by lymphocytes and plasma cells was always present in these sections. The intimal thickenings which overlay the parasitic tracts were composed of smooth muscle cells and elastic fibers, either longitudinally or circumferentially arranged. The underlying internal elastic lamella was for the most part intact, with only focal areas of interuption or duplication.

Parasitic migration was also felt to be responsible for a large, irregularly shaped gray plaque which was found in the arch of the aorta of a female golden cat (Felis temmincki). The diagnosis was suggested by the peculiar shape of the plaque and the presence of lymphocytes and plasma cells in the adjacent media and adventitia. This lesion was brightly stained by Sudan IV, and fine droplets of Oil Red-O positive material were distributed diffusely throughout its substance. Narrow, longitudinal, gray, serpiginous intimal thickenings, up to 2 cm in length, were seen in the lower thoracic aortas of several bears, but no other evidence for parasitism could be found, even after step-sectioning of several such blocks.

Multiple saccular aneurysms were found in the aortas of 2 black bears (Ursus americanus) one male and one female (Fig 3). The male was 16 years of age, and the female was also thought to be elderly. One bear was strangled accidentally during capture, the other was sacrificed during the course of an experiment. No other significant abnormalities were found at necropsy. The mouths of the aneurysms measured from 5 to 25 mm in diameter; they were confined to the lateral and posterior walls of the descending thoracic aortas. The intima overlying the base of the aneurysms was gray and thickened, and occasionally contained extensive, thin, plaque-like calcifications. No mural thrombi were observed. The study of representative sections revealed that all aneurysms were histologically similar, and apparently due to focal necrosis of the media. Although no acutely necrotic smooth muscle cells were seen, these cells were absent in large areas of the involved media, leaving sheets of thin hyalinized tissue containing sparse elongated nuclei. Elastic fibers at the

margins of the aneurysms were fragmented, closely aligned, and often clumped and coiled in tangled masses (Fig 4). Elastic fibers were absent in the center of the aneurysms. The affected media contained moderately numerous thin walled vascular channels. The overlying intima was greatly thickened and hyalinized, with foci of dystrophic calcification. Similar, although less extensive, medionecrosis was present adjacent to the aneurysms. The media was normal, however, in sections from the arch and abdominal portions of the aorta. Inflammatory cells were absent, but the walls of the small adventitial arteries adjacent to the aneurysms were markedly thickened. This thickening usually was due to masses of longitudinally aligned smooth muscle cells in the intima (Fig 5).

Pearly intimal plaques composed of musculo-elastic tissue similar to those of the Pinnipedia were found in the abdominal aortas and distal to branch orifices in 4 of 5 black bears, in 1 sloth bear (*Melursus ursinus*), and, to a lesser extent, in 1 spotted hyena (*Crocuta crocuta*), and 1 spotted cat (*Felis rubiginosa*).

Sudan positive plaques were seen in only 3 animals, one of which has been described above. The other 2 lesions were small papules, partially gray and partially pink, at the distal rim of the superior mesenteric artery and at the lateral rim of the left renal artery, respectively, in the 2 black bears with multiple aortic aneurysms. Faint Sudan positive flecks were noted at the distal rims of several branch orifices in the aortas of 1 spotted hvena and 1 sloth bear. Nonelevated Sudan positive patches with illdefined borders were present in the abdominal aortas of both of these animals, and in the same region in a male civet (Viverra civetta). Microscopically, the lipid in the latter 5 cases was Oil Red-O positive, and was dispersed in fine droplets throughout a normal appearing intima and adjacent media, or focally within a musculo-elastic plaque. The lipid droplets were occasionally clustered along elastic fibers, but were usually so diffusely scattered that it was difficult to tell whether they were intracellular or extracellular in location. No foam cells could be identified. Similar, but less extensive collections of finely dispersed droplets of Oil Red-O positive material were found in sections of the aortas of several other Carnivora. These accumulations of lipid usually occurred in normal intimal and medial layers, but were also present in musculo-elastic intimal thickenings. No doubly refractile crystals could be demonstrated. and no foam cells or amorphous lipid-rich zones characteristic of atherosclerosis were seen.

Diets

All felines consumed a diet consisting almost exclusively of meat,

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which was provided in single equal feedings 6 times weekly. In 1967, chicken replaced horsemeat for 3 of the weekly feedings; prior to that year horsemeat or ground beef was fed. Hyenas, badgers, bears, raccoons, and other Carnivora were fed single portions of horsemeat 5 times weekly; dog food daily. In 1967, 2 of the 5 horsemeat feedings were replaced by chicken each week. All carnivorous animals were given a multiple vitamin preparation and ground bonemeal in addition to the above described diets. The sea lion and seals were fed mackeral or smelt, with added vitamin B₁. Food thrown by the public was available to the large Carnivora and the seals and sea lions. Although the latter received primarily fish from public food dispensers installed for that purpose, the large Carnivora, and particularly the bears, were offered a variety of tid-bits including peanuts, popcorn, candy, and other items.

Postmortem Findings

The most common causes of death were trauma, various infectious diseases, and parasitic infestations. Unusual postmortem findings included a perforated duodenal ulcer in an adult male spotted hyena, achalasia in a young male harbor seal, and herniation with strangulation of the fundus and body of the stomach through a dilated diaphragmatic hiatus in a male golden cat. Two bears died with cancer; an adult male sloth bear with an adenocarcinoma of biliary duct origin, and an adult male black bear with an alveolar cell carcinoma of the lung. The latter animal also had disseminated granulomata involving the lungs and both adrenal glands. These lesions were hyalinized and calcified, and the causative agent could not be identified. There was no obvious connection between the degree of aortic disease and the pathologic findings at necropsy. However, the wide variety of species studied, and the relatively small number of individuals in any one species precluded a statistically valid analysis of the data in this respect.

Discussion

The medionecrosis observed in the 2 elderly bears was histologically very similar to that which occurs in man,^{33,34} although no cyst-like spaces were present. The process in the bears was also apparently more focal, resulting in multiple saccular aneurysms rather than the fusiform or dissecting aneurysms which are seen sometimes in humans with this disease. The pathogenesis of these lesions in the bears is obscure, and it was not possible to determine accurately whether degeneration of smooth muscle or elastic tissue was predominant. The fact that the abnormality was confined to the descending thoracic aorta is somewhat

against a stress and strain etiology, since hemodynamic forces are thought to be maximal in the arch of the aorta. Unfortunately, the blood pressure was not documented systematically during the life of either animal. The focal nature of the lesion, and the fact that parasite-induced aortic disease is common in Carnivora suggests a parasitic etiology. Finlayson.⁵ after an extensive study of arterial disease in mammals, birds. and reptiles dving in the London Zoo, has stated that aortic aneurysms in Carnivora probably are nearly always due to parasitism. However, step-sections through several blocks from aneurysms in the present 2 bears failed to reveal any change reminiscent of parasitic migration. Therefore, it is felt that these lesions represent exceptions to Finlayson's rule, which, of course, does not necessarily detract from its basic validity. The marked intimal thickening observed in the vasa vasorum near the areas of medionecrosis merits consideration, particularly since Schlichter, Amromin, and Solway 85 found similar changes in aortas with medionecrosis in humans. Cowan 38 also has reported thickening of the adventitial arteries of the aorta in association with medionecrosis, medial degeneration, or intimal plaques in several animal species.

The lesions found in the aortas of the Pinnipedia, except for the virtual absence of lipids, most closely resembled the early proliferative atherosclerotic lesions 37,38 of man. Plaques were found at the distal rims of branch orifices and were more numerous in the abdominal aortas. Their shapes and locations suggested that hemodynamic forces has been important in their formation. The lesions were composed almost entirely of smooth muscle cells, which in the larger plaques were sometimes arranged in layers, the deeper layer of cells oriented longitudinally, the outer circumferentially. The internal elastic lamella was intact beneath small plaques, and became progressively more fragmented as the thickness of the intimal lesion increased. Since lipids are thought to play an integral role in the development of the early proliferative atherosclerotic lesion in man, 39 it is puzzling that morphologically similar lesions occur in Pinnipedia in the absence of demonstrable lipid accumulations. Lindsay and Chaikoff 4 have stated previously that the deposition of lipid. although common, has very little to do with the pathogenesis of arterial disease in animals, and similar feelings have been expressed by Dahme. 40 While it is hazardous to compare the diseases of animals with those of man, the arteries of most mammalian species are structurally alike, and it seems significant that the presence of a pathogenic mechanism—accumulation of various lipid substances within the intima leading to proliferation of smooth muscle cells, etc.—thought to be important in the production of musculo-elastic intimal plaques in man cannot be docu-

mented in other animal species with similar arterial lesions.

Further insight into these problems may be obtained by comparing the findings in the aortas of the 45 Carnivora in the present study with the aortas of 86 hoofed mammals (Artiodactyla and Perissodactyla) from a previous study.²⁵ The proportion of aortas with musculo-elastic intimal plaques was approximately equal in the two groups; 15/45 for Carnivora, 26/86 for hoofed mammals. Sudan positive lesions, however, were noted in 42 of the 86 hoofed mammals, but in only 7 of the 45 Carnivora. Although additional small lipid accumulations were found in the aortas of several more Carnivora after microscopic examination, the hoofed mammal aortas were clearly predominant in this respect. Furthermore, the lipid accumulations presumably had been present for longer periods of time in the hoofed mammal aortas, since the aortas of 14 of 35 neonatal hoofed mammals were Sudan positive, whereas none of those of 17 neonatal Carnivora were stained by the dye. Therefore, although it is admittedly risky to make interspecies comparisons, it appears that another situation exists in which the amount of lipid present within an artery cannot be correlated positively with the amount of smooth muscle proliferation in the intima of that artery.

Atherosclerotic plaques sometimes occur in the aortas of hoofed mammals, but are quite rare in those of Carnivora. Perhaps the presence of more abundant lipid accumulations for longer periods of time in the arteries of hoofed mammals is responsible for this difference. However, musculo-elastic intimal plaques and atherosclerotic lesions appeared to develop independently of one another in the hoofed mammal aortas examined in this laboratory. Moreover, Likar et al have recently demonstrated microthrombi on the surface of, or within the intima of the left coronary artery in 28 of 51 Holstein-Frisian cows. The incorporation and subsequent breakdown of such thrombi within the vascular wall could certainly lead to atherosclerosis.

When atherosclerosis in man and animals is compared in a broad, general way, two major differences emerge: (1) man is much more severely affected, and (2) grossly visible mural thrombi are seen very frequently in human lesions, very rarely in animal lesions. The latter statement is well illustrated by the fact that not a trace of thrombotic material was found within the multiple aneurysms of the 2 bears described in the present report. It is therefore tempting to speculate that the severity of atherosclerosis in humans, and its mildness in animals, are due to the prevalence of mural thrombosis in the former, and to the paucity of mural thrombosis in the latter.

Summary

The degree of aortic arterial disease is documented in 45 Carnivora and 11 Pinnipedia dying in the Oklahoma City Zoo. Lesions were most prevalent in the Pinnipedia, and, except for the virtual absence of stainable lipid, were similar morphologically to the early proliferative atherosclerotic lesions of man. The fact that these musculo-elastic intimal plaques developed in the absence of stainable lipid accumulations seemed significant, possibly casting some doubt upon the hypothesis that lipids play an integral role in the pathogenesis of the early proliferative atherosclerotic lesion in man. This argument was further strengthened by the finding in this laboratory that the prevalence of musculo-elastic intimal plaques was equal in Carnivora and hoofed mammals, despite a far greater prevalence of lipid-containing lesions in the latter. Finanlly, the speculation was advanced that the well known difference between man and animals, in terms of atherosclerotic disease, is due to the frequency of mural thrombosis in man, and its scarcity in animals.

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[Illustrations follow]

Legends for Figures

Fig 1. Sea lion (Z. californianus), abdominal aorta. Section of small intimal plaque composed of smooth muscle cells. Internal elastic lamella is focally duplicated. H & E. \times 128.

Fig 2. Sea lion (Z. californianus), lower thoracic aorta. Section of large musculo-elastic intimal plaque. Internal elastic lamella is absent beneath central portion of lesion. Weigert's elastic stain. \times 20.

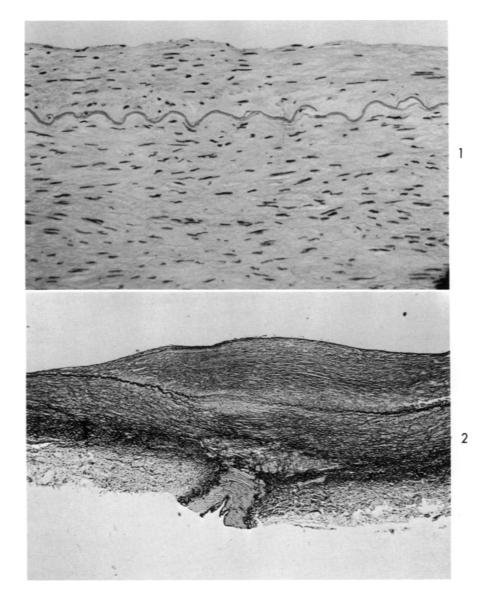




Fig 3. Black bear ($\emph{U. americanus}$). Thoracic aorta showing multiple saccular aneurysms.

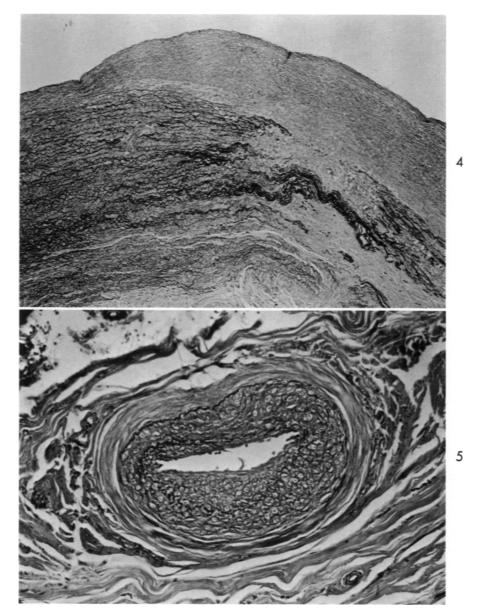


Fig 4. Black bear (U. americanus). Section through the rim of small aortic aneurysm showing loss of medial smooth muscle and elastic tissue. Overlying intima is considerably thickened by hyaline-like tissue. Weigert's elastic stain. \times 20.

Fig 5. Black bear (U. americanus). Section of adventitial artery adjacent to aortic aneurysm. Intima is remarkably thickened by longitudinally oriented smooth muscle cells. Internal elastic lamella is nearly intact; proliferated elastic fibers are present within lesion. Weigert's elastic stain. \times 128.